Physicochemical and Mechanical Effects of Commercial Silver Diamine Fluoride (SDF) Agents on Demineralized Dentin

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Purpose: To investigate the effects of four commercial silver diamine fluoride (SDF) agents on the chemical composition and microstructural properties of dentin, and its relation to the bond strength of two adhesives.

Materials and Methods: Ninety human molars were randomly divided into sound dentin (negative control), demineralized dentin (positive control), and four experimental groups (n = 15) according to the SDF treatments (Cariestop [Biodinamica Quimica y Farmaceutica], RivaStar 1 [SDI], RivaStar 2 [SDI], and Saforide [Tokyo Seiyaku Kasei]). ATR-FTIR, x-ray diffraction, and SEM techniques were employed to characterize the compositional, crystalline, and microstructural properties of the samples. The microtensile bond strength test evaluated the bonding performance of two adhesives in demineralized dentin treated with SDF agents.

Results: Regarding the chemical composition, all SDF-treated groups showed a significantly higher phosphate:organic matrix ratio than the demineralized dentin group ($p < 0.05$). The XRD analyses revealed that the crystallite size for hydroxyapatite crystals increased on the surface areas (deep, medium, and superficial dentin) for all experimental groups compared to demineralized dentin ($p < 0.05$). SEM images showed that the behavior of the agents used differs on each surface treated. Bond strength values were adversely affected with both adhesive systems in the four experimental groups ($p < 0.05$).

Conclusions: The application of SDF agents resulted in the formation of different crystalline phases of silver salts and the increase of mineralization of the pretreated demineralized dentin. However, SDF application showed a negative effect on the bond strength of the adhesives.

Keywords: adhesion, chemomechanical, dentin, mechanical tests, silver diamine fluoride.

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Dental caries is the localized destruction of susceptible dental hard tissues by the acidic by-products from bacterial fermentation of dietary carbohydrates.[8] Despite all the preventive measures available, caries remains one of the most prevalent chronic diseases worldwide. Traditional caries treatment consists of mechanical removal of the diseased tissue for subsequent restoration. Nevertheless, the development of novel dental materials and a better understanding of the caries process have led to the practice of minimally invasive dentistry.[27] From this perspective, the principles that guide the removal of carious tissue include delaying and minimizing the operative management of lesions, as well as reducing the pain or discomfort for patients.[31]

Morphologically, dentin consists of dentin tubules inside a matrix of intertubular dentin. The density and diameter of tubules decrease from the pulp to the outer edge of the cement or enamel, in such a way the areas of the dentinal tubules, peritubular and intertubular dentin vary dramatically between deep, medium and outer areas of the dentin.[28] From a compositional point of view, dentin consists of a

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main mineral phase, composed of carbonate-rich hydroxyapatite crystals, and an organic phase, which contains different extracellular proteins, such as collagen type I, proteoglycans, phosphoproteins, and sialoprotein.[28] The carious process leads to several chemical changes in dentin that include: lower mineral density; lower Ca:P ratio; decrease in magnesium and carbonate concentrations; and a partial dissolution of apatite crystals.[17] On the other hand, changes in the organic phase are associated with an alteration in the secondary structure of collagen and its distribution.[28]

Silver diamine fluoride (SDF) is a topical agent indicated to treat and prevent tooth decay. SDF consists of a colorless alkaline solution containing silver and fluoride, which form complex compounds with ammonia.[43] SDF-based treatments have been used for arresting caries in children since the early 1960s in Japan.[39] The main disadvantage of the SDF application is surface staining due to the oxidation of free silver ions. The use of potassium iodide (KI) produces silver iodide, a white reaction agent that ameliorates the black stain of the dentin surface.[15] Multiple investigations have more recently proven the clinical uses and advantages of SDF, including its preventive and cariostatic action in children[4] and the elderly,[12,30] as a desensitizing agent[15] and its antimicrobial effect as an irrigant in endodontics.[39] Additionally, in vitro studies have also shown the inhibitory effects on metalloproteinases,[25] cathepsins,[23] and the preventive action of collagen degradation.[24]

Adhesion in dentin continues to be a challenge which, despite current technology, is not fully achieved due to the heterogeneity of the dental substrates. It has been reported that the bond strength on affected dentin is lower than on sound dentin, regardless of the type of adhesive used.[28] However, in vitro studies report that etch-and-rinse adhesives perform better than the self-etching adhesives on affected dentin.[3] The use of SDF has increased due to the low cost, easy technique, and growing number of commercially available agents. Nevertheless, the restorative protocol for these SDF-treated dentin surfaces is not completely clear. Previous studies suggest that dentin pretreated with SDF and KI (potassium iodide) does not interfere with the bond strength of glass-ionomer cement to dentin.[41,42] However, the bond strength does not seem to be as satisfactory when such cavities are restored with composites. Preliminary studies have provided evidence that dentin pretreated with SDF/KI negatively affects the bond strength of immediate composite restorations.[15,20,40] Nevertheless, other investigations suggest that SDF pretreatment does not affect the bond strength of resin composite to dentin.[33,38] Overall, there is no consensus on the immediate effect of SDF on the bonding of adhesive materials.[10,13]

It is noteworthy that adhesives are very technique sensitive; any modification on the dentin surface or variation in the protocols may affect the adhesion performance. In this regard, this study aims to investigate the effects of commercial SDF treatments on the chemical composition and microstructural properties of dentin, and its relation to the bond strength of the adhesives. For this purpose, two hypotheses were tested: (1) the application of four commercial SDF agents affects the chemical and microstructural properties of demineralized dentin surfaces; (2) the application of these SDF agents on demineralized dentin treated with etch-and-rinse and self-etching adhesives affects the bond strength of adhesives.

MATERIALS AND METHODS

The current research received approval by the local Ethics Committee on Human and Animal Research (#1020-2020). Ninety non-carious human permanent molars were stored in 0.1% thymol solution at 4ºC before preparation.

Specimen Preparation

The tooth roots were separated from the crowns 2 mm below the cementoenamel junction, and the occlusal enamel was removed using an Isomet 11/1180 microtome (Buehler; Lake Bluff, IL, USA) with a diamond disk XL 12205 (Benetec; London, UK). The exposed dentin surface was wet-polished with 500-grit silicon carbide (SiC) paper (Lunn Major, Struers; Ballerup, Denmark) to create a continuous, flat dentin surface. The pulp chambers were cleaned and filled with a bonded resin composite (Coltene-Whaledent; Altstätten, Switzerland) to act as support. Subsequently, the teeth were rinsed with distilled water to remove the remaining debris.

Artificial Caries Induction (pH-Cycling)

The caries-affected dentin lesions were created by a pH-cycling procedure modified by Marquezan et al.[21] The specimens were immersed in 10 ml of a demineralizing solution containing 2 mM $CaCl_2$, 2.2 mM NaH_2PO_4, and 50 mM acetic acid adjusted to a pH of 4.8 for 8 h. Thereafter, samples were immersed in 10 ml of a remineralizing solution containing 1.5 mM $CaCl_2$, 0.9 mM NaH_2PO_4, and 0.15 M KCl adjusted to a pH of 7.0 for 16 h. Each specimen was cycled for 14 days, and the solutions were changed daily. pH cycling was performed at room temperature without agitation. All experimental groups were subjected to the same pH-cycling procedure; the sound dentin group was not.

Experimental Design

The teeth were randomly divided into sound dentin (n = 15, negative control), demineralized dentin (n = 15, positive control), and four experimental groups (n = 15 per group) according to the silver diamine fluoride (SDF) agents applied: Cariestop (Biodinâmica Química y Farmacêutica; Ibipora, Brazil); RivaStar 1 (capsule 1) and RivaStar 2 (capsule 1+2) (both SDI; Bayswater, Victoria, Australia); and Saforide (Tokyo Seiyaku Kasei; Osaka, Japan). The dentin surfaces were treated with the commercial SDF agents shown in Table 1, including the trade-name, manufacturer, chemical composition, and application procedures. Subsequently, 5 teeth from each group were used for chemical, microstructural, and morphologic analyses, and the remaining 10 teeth were used for mechanical testing.

Table 1 Materials, manufacturers, chemical compositions, and application procedures of the SDF solutions and adhesives

Material	Composition	Application
Cariestop 30% (Biodinâmica Química y Farmacêutica; Ibipora, Brazil)	Ammonium hydroxide, silver nitrate, hydrofluoric acid, deionized water	Apply with a brush for 2–3 min. Rinse.
Riva Star (SDI; Bayswater, Victoria, Australia)	Capsule 1: silver fluoride, ammonia solution Capsule 2: Potassium iodide	Capsule 1: Apply the solution with a brush to the treatment site only. Capsule 2: Apply a generous amount of the solution to the treatment site until the solution turns clear.
Saforide (Tokyo Seiyaku Kasei; Osaka, Japan)	Ammonium hydroxide, silver nitrate, hydrofluoric acid, water	Apply with a brush for 3 min. Rinse.
Clearfil SE Bond 2 (Kuraray Noritake; Tokyo, Japan)	Primer: MDP, HEMA, hydrophilic dimethacrylate, dl-camphorquinone, accelerators, water Bond: MDP, bis-GMA, HEMA, dl-camphorquinone, accelerators, silanated colloidal silica	Primer: Apply for 20 s. Dry for 5 s. Bond: Apply and light cure 10 s.
Gel etchant (Kerr; Orange, CA, USA) Optibond FL (Kerr)	37.5% phosphoric acid Primer: HEMA, GPDM, PAMM, ethanol, water, photo-initiator Bond: TEG-DMA, UDMA, GPDM, HEMA, bis-GMA, filler, photo-initiator	Apply for 15 s. Rinse for 15 s. Dry. Primer: Apply for 15 s. Dry for 5 s Bond: Apply for 15 s. Dry for 3 s. Light cure 20 s.

MDP: 10-methacryloxyloxydecyl dihydrogen phosphate; bis-GMA: bisphenol A diglycidylmethacrylate; GDMA: glycerol dimethacrylate; HEMA: 2-hydroxyethyl methacrylate; UDMA: urethane dimethacrylate; PAMM: phthalic acid monoethly methacrylate; TEG-DMA: triethylene glycol dimethacrylate.

Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR)

Three teeth per group were analyzed by ATR-FTIR to assess potential changes in the chemical composition of dentin after SDF treatment. Samples were analyzed using an FTIR JASCO 6200 spectrometer equipped with a diamond-tipped ATR accessory (ATR Pro ONE, Jasco; Easton, MD, USA). The spectra were recorded at a resolution of 2 cm^{-1} with 32 accumulations with a spectral range of 600-4000 cm^{-1} in absorption mode. Peak overlapping was resolved and their integrated areas were measured using curve-fitting software (Peakfit v4.12, Systat Software; San Jose, CA, USA). The second derivative method was used to resolve the peak calculations within the spectrum region. The degree of smoothing was adjusted to 10% (Savitzky-Golay algorithm) and a mixed Gaussian-Lorentzian function was used to adapt the contours of the peaks within the region. A variation of the maximum amplitude and position, 5% and ± 2 cm^{-1}, respectively, was allowed for each peak during fitting. The curve fit was accepted when r^2 achieved values higher than 0.95. The relative amounts of amides (mainly corresponding to collagen), phosphate, and carbonate molecular groups in the dentin samples were determined from the peak area of the absorption bands associated with each component.[2,18,34] The following parameters were calculated to describe dentin compositional properties: 1. the relative amount of mineral to organic matrix (PO_4/Amidel) determined as the ratio of the main phosphate (v_1, v_3 PO_4; 900-1200 cm^{-1}) to the Amidel area ratio (collagen type I; 1690 cm^{-1}); 2. the carbonate substituted to the mineral content (CO_3/PO_4) was determined as the ratio between the carbonate substituted (v_3 CO_3; 1390-1440 cm^{-1}, type B substitution) to the main phosphate band area (900–1200 cm^{-1}); 3. the crystallinity index (CI) was determined as the ratio between phosphate sub-band areas at 1030 cm^{-1} (highly crystalline apatite phosphates) to 1020 cm^{-1} (poorly crystalline apatite phosphates).[18,26,34]

X-ray Diffraction (XRD)

One tooth from each group was used to study the crystalline characteristics of the phases formed during treatments. Two-dimensional x-ray diffraction (2D-XRD) patterns were obtained by using an x-ray diffractometer (Bruker D8 Discover, Bruker; Billerica, MA, USA) equipped with a 2D detector (Dectris Pilatus 3 100K-A; Baden-Dättwil, Switzerland). For the diffraction experiments, the working conditions were: Cu Kα (λ = 1.5418 Å), 50 kV, and 30 mA, with a pinhole collimator of 0.5-mm diameter. The analyzed spots (ie, locations on the sample surface) were determined employing an optical microscope equipped with a laser reference. Diffraction patterns were obtained from three different locations on the dentin surface according to its depth: superficial, medium, and deep. The 2D-XRD patterns were registered at a 2θ scanning angle range from 20 to 60 degrees, considering 19 steps and 40 s/step. The intensities concentrated in arcs within the Debye diffraction rings (corresponding to specific diffraction lines) were inte-

Table 2 Mean (SD) of the compositional parameters analyzed by ATR-FTIR

Group	Phosphate to matrix ratio	Carbonate to phosphate ratio	Crystallinity Index (CI)
Sound dentin	3.085 (0.889)[a]	0.164 (0.037)[a]	1.262 (0.207)[a]
Demineralized dentin	0.809 (0.247)[b]	0.171 (0.027)[a]	1.641 (0.202)[b]
Cariestop	1.102 (0.338)[c]	0.137 (0.041)[b]	1.654 (0.255)[b]
RivaStar 2	2.508 (1.561)[ad]	0.109 (0.072)[ad]	0.918 (0.296)[e]
RivaStar 1	1.470 (0.426)[d]	0.107 (0.032)[cd]	1.387 (0.193)[d]
Saforide	1.863 (0.924)[cd]	0.120 (0.051)[d]	1.294 (0.232)[ad]

Different letters indicate significant differences in columns.

grated to obtain a unidimensional 2θ scan. The XRD patterns were identified by comparing them to the JCPDS data cards using XPowder software (www.xpowder.com, Spain).[22]

The crystallite size for hydroxyapatite of dentin mineral and other crystalline phases (ie, SDF reaction compounds) was determined by measuring the full width at half maximum (FWHM) of the diffraction peaks displayed in the 2θ pattern. For this purpose, the Debye-Scherrer equation was used to estimate crystallite size:[6]

$$d = K\lambda / \beta \cos \theta$$

where d is the mean size of the crystallites (expressed in nm), λ is the wavelength of the x-ray source, K is Scherrer's constant, assumed as a factor K = 0.89, and β is the FWHM of the line broadening for specific diffraction reflections. It should be noted that the determination of crystallite size using XRD techniques corresponds to the volume-averaged measurement of the crystalline domains.

Scanning Electron Microscopy (SEM/EDS)

One sample per group was used to observe the morphological changes in the dentin surface and tubular occlusion. Each sample was sectioned into three specimens to observe the dentin surface, SDF treatment with 37.5% phosphoric acid, and SDF treatment with Clearfil SE Bond 2 (Kuraray Noritake; Tokyo, Japan). All samples were fixed in glutaraldehyde solution after being rinsed with PBS. Samples were dehydrated in ascending ethanol series (50%, 70%, 90%, 96%). Subsequently, samples were sputter-coated with gold using a Nanotech Polaron-Semprep2 (Polaron Equipment; Watford, UK) and observed at 15,000X under 20kV SEM (S-510, Hitachi; Tokyo, Japan) equipped with an EDS detector to assess.

Microtensile Bond Strength Test (μTBS)

The teeth of the experimental groups and controls (n = 10) were further assigned to one of the bonding subgroups, etch-and-rinse adhesive (OptiBond FL, Kerr; Orange, CA, USA) and 2-step self-etching adhesive (Clearfil SE Bond 2, Kuraray Noritake). Both adhesives were applied (n = 5, respectively) according to the manufacturer's information. Finally, a block of composite was built up to a height of 8 mm by applying 2-mm layers of Synergy D6 resin composite (Coltene-Whaledent). Each layer was light cured for 20 s with a LED (Bluephase, Ivoclar Vivadent; Schaan, Liechtenstein), which was verified to have a light output of 1200 mW/cm^2, as indicated by the unit's radiometer. The samples were stored in distilled water for 24 h.

Samples were serially sectioned in the "x" and "y" directions to obtain 1 x 1 mm sticks through the adhesive/tooth. The specimens were inspected to confirm the absence of microcracks and their dimensions were measured. The number of premature failures (PTFs) per tooth during specimen preparation were recorded. The sticks were fixed to a modified Bencor Multi-T testing support using cyanoacrylate (Super Glue 3, Loctite Ibérica; Barcelona, Spain). The microtensile testing was performed with an Instron 3345 machine (Instron; Norwood, MA, USA) with a 500 N load cell at a crosshead speed of 0.1 mm/min. The failure mode was examined with a light microscope (Olympus; 50X magnification) and classified as cohesive (debonding occurred inside the dentin), adhesive (debonding between dentin and adhesive layer) and, mixed (fracture line combination). Cohesive failures inside composite did not occur.

Statistical Analysis

All statistical analyses were performed using the software SPSS 24.0 (SPSS; Chicago, USA). The normal distribution of the variables was checked by Shapiro-Wilk test. Since the variables did not follow normal data distribution, non-parametric statistical test were employed for analysis. For the compositional parameters analyzed in ATR-FTIR and the bond strength values, the Kruskal-Wallis nonparametric test was used followed by an intergroup analysis with the Mann-Whitney U-test. The chi-squared test was used to compare failure modes. A level of significance of $p < 0.05$ was established.

RESULTS

Attenuated Total Reflectance Fourier Transform Spectral Analysis (ATR-FTIR)

The mean and standard deviation values for compositional parameters determined by ATR-FTIR are shown in Table 2. After the pH-cycling procedure, the phosphate to organic

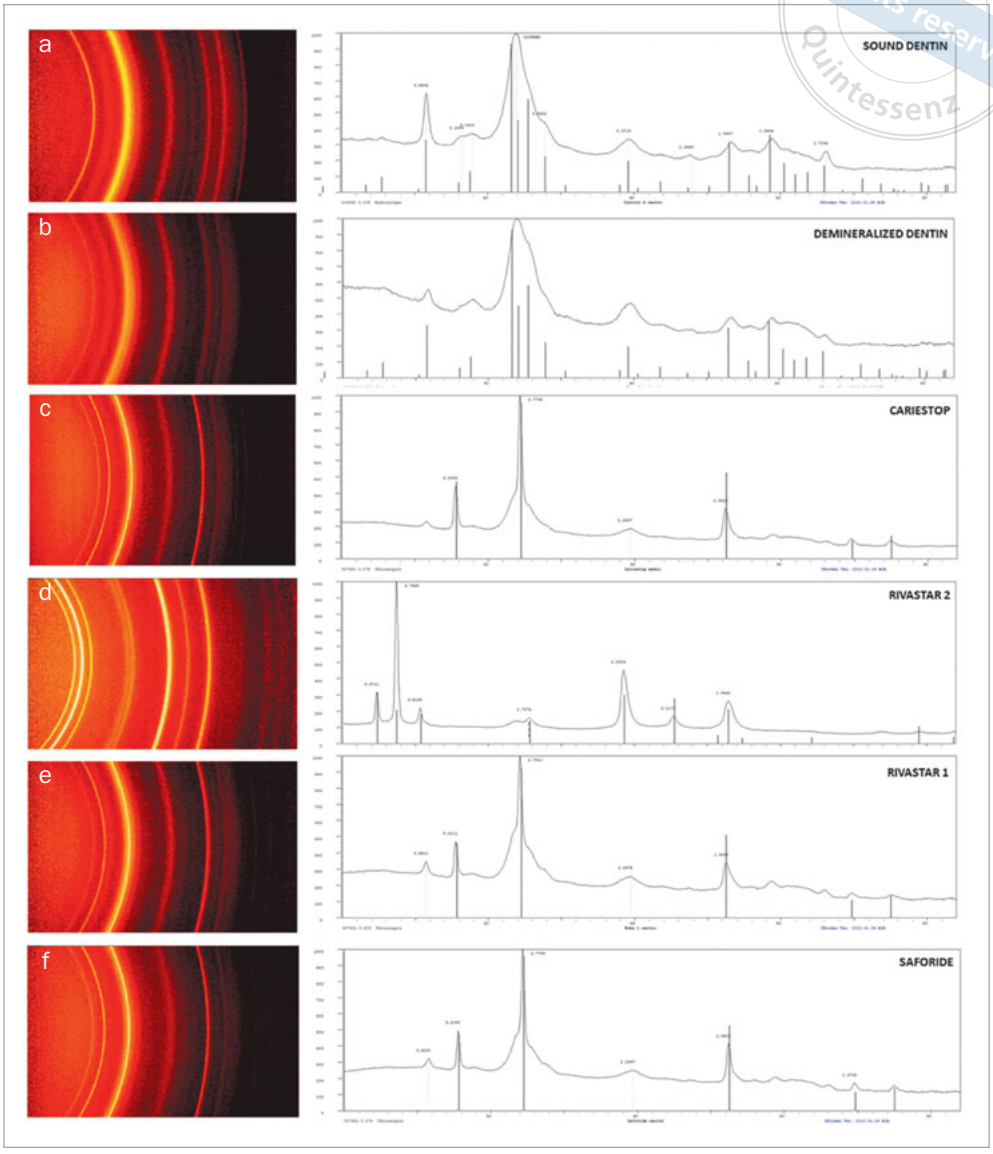

Fig 1 2D-XRD patterns showing the Debye–Scherrer rings (left column) and integrated 2Theta scan (right column) for (a) dentin control; (b) demineralized dentin and SDF treatments; (c) Cariestop; (d) RivaStar 2; (e) RivaStar1, (f) Saforide. Vertical lines represent the peak positions reference for hydroxyapatite (a, b), AgCl (c, e, f), and AgI (d) diffraction patterns.

matrix ratio decreased significantly compared to the sound dentin group ($p < 0.05$). In the SDF treatment groups, the phosphate to organic matrix ratio was significantly higher than in the demineralized dentin group ($p< 0.05$). On the other hand, the carbonate to phosphate ratio decreased significantly in three SDF groups (ie, RivaStar2, RivaStar1, Saforide) with respect to sound and demineralized dentin groups. The Crystallinity Index (CI) was significantly higher in demineralized dentin and Cariestop compared to all experimental groups ($p < 0.05$). However, RivaStar 1 and Saforide showed similar CI values than sound dentin, while RivaStar 2 presented the lowest values.

X-ray Diffraction (XRD) Analysis

The crystallinity properties of the dentin mineral and SDF compounds were studied by means of 2D-XRD. Figure 1 shows the 2D-XRD patterns and the integrated 2Theta scan (unidimensional pattern) for the sound and demineralized dentin (Figs 1a and 1b). The XRD peaks observed in sound dentin, associated with the hydroxyapatite crystal diffraction pattern (reference vertical lines), were sharper than those subjected to pH-cycling (demineralized dentin). Figure 1 also shows the 2D-XRD patterns and the integrated 2Theta scans of the different SDF treatments (Figs 3c to 3f). The diffraction patterns show highly crystalline phases, with bright, sharp diffraction peaks, formed as a result of the treatments in the demineralized dentin surface. Through the identification of the diffraction lines, the synthetic crystalline phases formed by SDF treatments, correspond to silver chloride (chlorargyrite, AgCl; cubic structure, $\alpha = 5.554$ Å) for RivaStar 1, Cariestop and Saforide agents, and silver iodide (iodoargyrite, AgI, hexagonal structure, $\alpha = 4.580$ Å,

Table 3 Full width at half maximum (FWHM) and crystallite size (d) of hydroxyapatite (HAp) in sound and demineralized dentin (including SDF treatments except RivaStar 2), silver chloride (ClAg) and silver iodide (IAg) in SDF treatments

		FWHM	d (nm)	FWHM	d (nm)
		HAp – 002		ClAg – 111	
Sound dentin	Deep	0.387	20.85		
	Medium	0.415	19.44		
	Superficial	0.401	20.12		
Demineralized dentin	Deep	0.585	13.79		
	Medium	0.663	12.17		
	Superficial	0.633	12.74		
Cariestop	Deep	0.345	23.38	0.223	36.32
	Medium	0.522	15.46	0.289	28.02
	Superficial	0.483	16.70	0.302	26.82
				IAg – (100)	
RivaStar 2	Deep	N.d.	Nd	0.23	34.85
	Medium	N.d.	Nd	0.281	28.52
	Superficial	N.d.	Nd	0.328	24.44
RivaStar 1	Deep	0.391	20.63	0.264	30.68
	Medium	0.525	15.37	0.329	24.62
	Superficial	0.428	18.85	0.314	25.79
Saforide	Deep	0.413	19.53	0.243	33.33
	Medium	0.55	14.67	0.332	24.39
	Superficial	0.549	14.69	0.375	21.60

Nd = not detected.

c = 7.510 Å) for RivaStar 2. These diffraction patterns also show lower and broader intensity peaks corresponding to hydroxyapatite diffraction lines (eg, 002 diffraction peak at 25.92-degree 2Theta position) related to dentin mineral.

Crystallite size measurements (ie, estimation of the size of the coherently scattering domains of crystals) for the mineral dentin and the SDF treatments are reported in Table 3. The crystallite size of the dentin mineral was determined by measuring the full width at half maximum (FWHM) for the 002 diffraction line (ie, corresponding to the c-axes) for hydroxyapatite crystal. Crystalline size (d) was determined at three distinct dentin areas distinguishable by their morphology, such as superficial, medium, and deep dentin. The results indicated that the crystallite size of hydroxyapatite crystals in demineralized dentin decreased compared to sound dentin in the three areas. The d values for the synthetic crystalline phases in SDF treatments corresponding to silver chloride (ClAg; RivaStar 1, Cariestop and Saforide) and silver iodide (IAg; RivaStar 2) were determined from the 111 and 100 diffraction peaks, respectively. After applying the SDF agents, the crystallite size of the hydroxyapatite crystal increased in all experimental groups and areas studied (except for RivaStar 2, not detected), compared to the demineralized dentin. The deep dentin area showed the largest increase in the crystallite size for both hydroxyapatite crystals and synthetic crystalline phases of ClAg and IAg in SDF-treated groups.

SEM and EDX Analysis

Figure 2a shows a typical sound dentin surface, while Fig 2b presents a wet-polished demineralized dentin surface covered with a thin smear layer. All tested SDF/KI treatments produced a precipitate on the dentin surfaces (Figs 2c to 2f). After 37.5% phosphoric acid application, then rinsing and drying, it was observed that the smear layer was removed, exposing the dentinal tubules of the demineralized dentin surface (Fig 2h). While in the SDF/KI treated groups, the precipitate appeared to be partially removed (Figs 2l to 2L). Application of the self-etching priming solution Clearfil SE Bond 2 on the treated groups with SDF/KI revealed a surface film of SDF and particles within the dentin tubules (Figs 2o to 2r). The particles from the SDF/KI treated surfaces were analyzed by EDX spectra. The EDX analysis of these small crystals indicated the presence of Ca, P, Mg, Ag, Cl on all treated samples, the presence of I was only detected in the RivaStar 2 group.

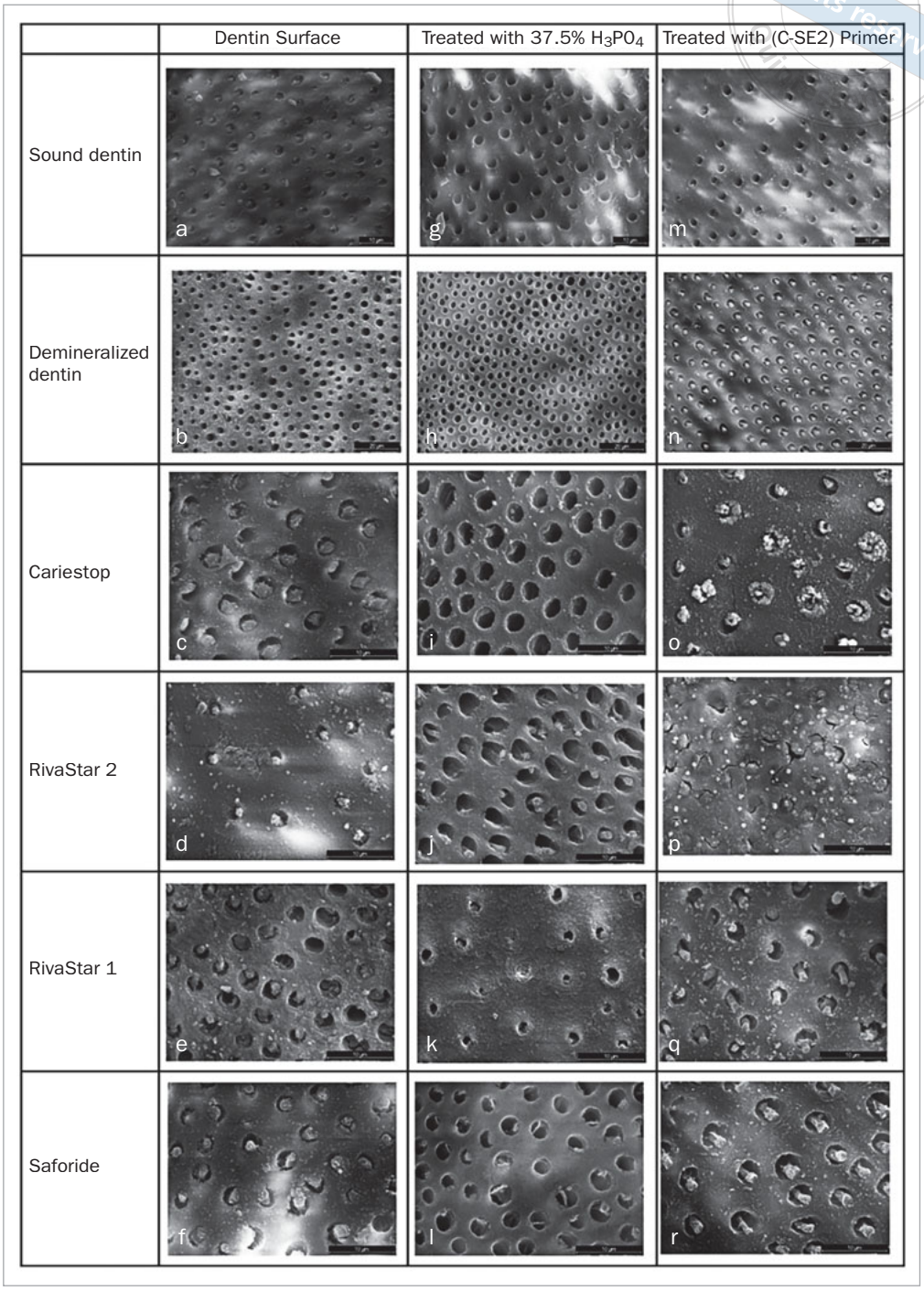

Fig 2 SEM images of dentin surface treatment with SDF agents (c–f), SDF agents and 37.5% phosphoric acid (i–l), SDF agents and Clearfil SE Bond 2 (C-SE2) primer (o–r).

Microtensile Bond Strength Test (μTBS)

The results of the μTBS test are summarized in Table 4. The pre-testing failures (PTFs) were replaced by a computer-determined random value between 0 MPa and the lowest value measured in the specific experimental group (1). All the specimens in the RivaStar 1 and RivaStar 2 groups treated with Clearfil SE Bond 2 recorded an average value of zero due to PTFs. The highest values were observed for the sound dentin group with both adhesives systems, Optibond FL and Clearfil SE Bond 2. After the application of the SDF agents, a significant drop ($p < 0.05$) was observed in the bond strengths with respect to the sound and demineralized dentin groups. Furthermore, there were significantly lower bond strengths ($p < 0.05$) in all groups using Clearfil SE Bond 2 self-etching adhesive compared to the groups using OptiBond FL etch-and-rinse adhesive. Considering the

Table 4 Microtensile bond strength means (SD)

Group	N	Optibond FL	Clearfil SE Bond 2
Sound dentin	49	37.6 (10.4)^{Aa}	29.9 (10.4)^{Ba}
Demineralized dentin	48	31.4 (6.6)^{Ab}	20.7 (11.1)^{Bb}
Cariestop	66	15.1 (9.2)^{Ad}	9.6 (6.9)^{Bc}
RivaStar 2	62	7.5 (4.4)^{e}	PTF
RivaRiva 1	65	10.1 (5.5)^{e}	PTF
Saforide	52	23.2 (8.2)^{Ac}	8.03 (4.3)^{Bc}

N: number of samples. PTF: pre-test failure. Values expressed in MPa. Different capital letters indicate significant differences in rows. Different lowercase letters indicate significant differences in columns.

four SDF treatment groups, Saforide showed the highest and lowest bond strengths with OptiBond FL (23.2 MPa) and Clearfil SE Bond 2 (8.1 MPa), respectively. The groups RivaStar 1 and RivaStar 2 showed the lowest bond strengths with Optibond FL (10.1 and 7.5 MPa, respectively). The modes of failure for all groups are summarized in Fig 3. Two failure modes were identified: adhesive and mixed. Most of the bond failures were adhesive in all the groups treated with SDF. Mixed fracture modes were identified in sound and demineralized dentin and in Cariestop with Optibond FL. No cohesive failures were observed in any group. There was a high incidence of PTFs in the four SDF treatment groups. All specimens in the RivaStar 1 and RivaStar 2 groups with the self-etching adhesive Clearfil SE Bond 2 failed prematurely and were excluded from the statistical analysis.

DISCUSSION

The results of the current study showed that SDF treatment causes different physicochemical alterations in the pretreated demineralized dentin surface which compromised immediate bond strength. With respect to the first hypothesis, pH cycling and the subsequent application of commercial SDF agents altered the mineral composition and the crystalline characteristics of dentin. Additionally, regarding the second hypothesis, the application of the SDF treatments to demineralized dentin surfaces with etch-and-rinse and self-etching adhesives had a negative effect on the bond strength. It can be assumed that the immediate restoration of these surfaces with all the tested SDF commercial agents may lead to failure due to lack of adhesion, which can result in further carious lesions.

The chemical composition of SDF-treated dentin was evaluated by ATR-FTIR spectroscopy. The dentin specimens were initially demineralized by pH cycling to simulate the caries lesion, resulting in a decrease in the phosphate:organic matrix ratio (related to the degree of dentin mineralization) and an increase in the crystallinity index. pH cycling dissolved the mineral components of dentin by removing the phosphate groups of lower crystallinity (associated with higher solubility), while the organic component (mainly collagen type I) remained unaltered.[7] After SDF treatment, the mineral components recovered, but not to the ad integrum state of sound dentin. Furthermore, it was previously shown that collagen degradation in dentin was partially inhibited by SDF solutions.[44] The maintenance and stability of dentin collagen during remineralization is essential, as it acts as a scaffold for mineral precipitation.[11] The SDF solutions provide an alkaline environment that promotes physicochemical interactions between the phosphate groups and dentin collagen.[23] Once phosphate groups attach to collagen, they contribute to the formation of Ca-ion binding sites, thus facilitating the nucleation of apatite.[29] This mechanism can also be coupled with the reaction of PO_4^{3-} ions from the demineralized dentin and Ag^{2+} ions present in the SDF agents, forming a silver phosphate (Ag_3PO_4) phase. However, this Ag_3PO_4 crystalline phase is unstable and reduces rapidly to form other metal compounds,[19] which can be identified by their crystalline structures (further discussed below). As a result, the studied SDF treatments promoted a remineralization effect on the inorganic content (mainly phosphate groups), increasing dentin mineral deposition, which was further supported by the inhibitory action on the degradation of the organic matrix.[25]

Several factors are involved in remineralization by SDF which promote apatite crystallization. During this process, the mineral residue after pH cycling may favor the heterogeneous nucleation of subsequent phases, and also act as a reservoir of available calcium and phosphorus ions, which in turn promote the precipitation of other crystalline phases on the dentin surface.[43,44] On the other hand, as previously mentioned, the structure of collagen serves as a scaffold for mineral deposition in dentin. The reaction of SDF compounds with mineral dentin lead to the formation of silver phosphate (Ag_3PO_4) and calcium fluoride (CaF) phases.[44] Calcium fluoride is highly soluble and easily removed from dentin surfaces during rinsing.[19] Moreover, silver oxides can react with solutions of alkali chlorides to form silver chloride (AgCl) crystals,[24] as was detected with XRD in this study. Additionally, one of the SDF agents used (RivaStar2)

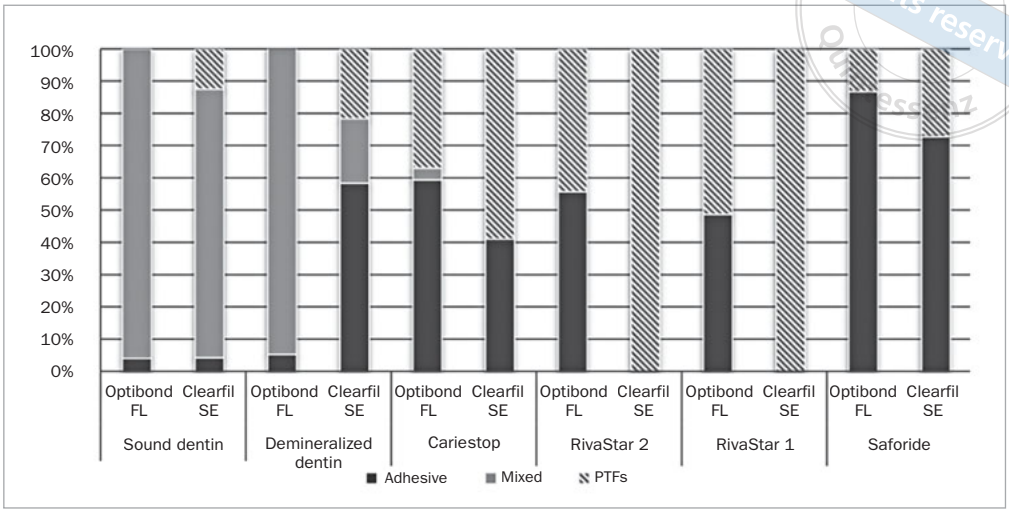

Fig 3 Distribution of failure modes by SDF treatment and adhesives (Clearfil SE Bond 2 abbreviated as Clearfil SE).

contains potassium iodide (KI), the presence of which promotes the crystalline precipitation of silver iodide (AgI). The formation of this silver-iodide crystalline phase (visible as a bright yellow solid) reduces the excess of silver ions that give rise to the typical black staining in other treatments with SDF agents.[14] These silver nanoparticles present high cytotoxicity and antibacterial properties, providing long-lasting protection against new caries formation.[36] Furthermore, the silver crystalline phases (AgCl and AgI) distributed on the dentin surfaces showed crystallite sizes ranging from 21 to 36 nm. On the other hand, apatite crystals reduced the crystallite size in demineralized dentin compared to sound dentin. Subsequently, the remineralization by SDF precipitated HAp crystals with crystallite sizes close to those of sound dentin. In addition, a correlation between crystallite size and dentin depth was observed for both the apatite and silver-salt phases, showing higher values in deep dentin than in surface and middle dentin areas. This relationship could be attributed to the increase in dentin tubule diameter and lower mineralization in the deep dentin zone,[28] altering the processes of dissolution and precipitation of the crystalline phases. Consequently, it can be assumed that the absorption of SDF into demineralized dentin does not occur homogeneously in depth. These compositional and microstructural factors that control the reaction mechanisms of SDF agents on the dentin surfaces are key to bonding quality, directly affecting the mechanical properties during restoration.

In the present study, SEM observations confirmed the presence of silver deposits after the application of the different SDF agents. Other studies reported similar findings, showing silver deposits within dentinal tubes.[15,16] However, the behavior of the agents used differs for each surface treated. This phenomenon can be seen in the RivaStar 1 and RivaStar 2 groups, which showed a more SDF deposits on their surfaces than on those of the other treated groups. When dentin samples were acid etched, the acid infiltrated the dentinal tubules and partially removed the SDF deposits. On the other hand, after the application of Clearfil SE Bond 2 Primer, an irregular surface was observed, showing occlusion of the dentinal tubules especially in the Cariestop and RivaStar 1 groups. These findings provided supporting evidence that SDF treatment interferes with the microstructural properties of demineralized dentin and the performance of the adhesives. The elemental analysis (EDX) indicated the presence of Ca, P, C, and Cl in all samples. However, the Ag content was detected only in the SDF treated groups and I (iodine) content was only observed in the RivaStar2 group. The presence of Cl is related to the high solubility salts of the demineralizing and remineralizing solutions used to create artificial caries.

The mechanical properties of dentin are influenced by its chemical and microstructural characteristics.[35] The application of SDF led to a decrease in the bond strength of both adhesives, which is consistent with the results observed by Soeno et al[40] and Kucukyilmaz et al,[16] who found that the reaction of SDF with the hydroxyapatite and proteins of the demineralized dentin surface prevents the infiltration of adhesive monomers. On the other hand, the application of SDF/KI to sound dentin did not adversely affect the bond strength of resin restorations.[33,38] This difference in bonding behavior can be explained by the morphological differences between demineralized and sound dentin substrates. The results obtained in our study were likely due to the increased penetration of free silver ions from the SDF treatments into a demineralized dentin lesion, as compared to sound dentin.[37] In this regard, free silver ions have been observed to interfere with adhesion by preventing resin impregnation of demineralized dentin tubules.[16,20] Between the SDF agents employed in the current study, RivaStar 1 and RivaStar 2 showed the lowest bond strengths, with a high percentage of PTFs. This alteration may be attributed to the application protocol, not including the rinsing step before the bonding procedures as described by the manu-

facturer. This presupposition may be in accord with a previous study by Lutgen et al,[20] which demonstrated a correlation between the negative effect on bonding and the SDF application protocol; rinsing after SDF application led to improved bond strength compared to non-rinsed groups. Furthermore, Koizumi et al[15] demonstrated that demineralized dentin surfaces treated with SDF/KI and subsequently bonded showed a significant decrease in bond strength, which was more pronounced with the self-etching than the etch-and-rinse adhesives.

Furthermore, the demineralized dentin group showed significantly lower bond strengths particularly using a self-etching adhesive (Clearfil SE Bond 2). These results are consistent with Ceballos et al,[3] who observed a higher bond strength with etch-and-rinse adhesives than with with self-etch adhesives on sound and demineralized dentin. Our findings showed that Optibond FL had the highest bond strengths compared with ClearFil SE Bond 2 in all four SDF treatment groups. The highly alkaline and temporally stable pH of SDF agents[5] may create a stabilization problem with self-etching adhesives, since acid monomers from the adhesive may not be acidic enough to infiltrate the dentin surface. As previously stated, the subtle difference in HAp crystallite sizes observed in all experimental groups critically influences the physicochemical properties (solubility rates) of the mineral at the dentin surface. The modification of these HAp crystalline characteristics may compromise the interaction of the adhesive in dentin by altering the formation of the hybrid layer. Therefore, the different mechanical responses, in terms of bond strength, may be related to the alteration of the structural characteristics of the dentin surface provoked by the treatment with SDF and adhesives. Accordingly, SEM images corroborated the lack of impregnation of the dentinal tubes and higher amounts of SDF deposits when using self-etching adhesives. The marked reduction in the bonding ability of the ClearFil SE Bond 2 self-etching adhesive could be attributed the fact that it contains 10-MDP monomer, considered as one of the effective functional monomers for dentin bonding.[32] This monomer reacts with Ca ions to form calcium salts, crucial for the proper functioning of this adhesive.[9] Consequently, it can be assumed that silver ions of the SDF agents interfere with the available calcium from the demineralized dentin, thus affecting the bonding mechanisms of self-etching adhesives and generating a weak hybrid layer. Regarding the etch-and-rinse adhesive, the superior performance of Optibond FL may be related to the application of phosphoric acid, which removed part of the SDF/KI layer that precipitated onto the dentin surface, as seen clearly in the SEM images.

Despite some limitations in the mechanical tests, this in vitro study shows that the application of SDF agents increases the degree of mineralization and crystalline properties of previously demineralized dentin. On the other hand, the use of SDF agents leads to a reduction in bond strength, especially using a self-etching adhesive. The clinical implications are relevant, as the immediate restoration of these cavities could result in premature restoration failure. Further research on application protocol is needed to determine the most suitable protocol to combine SDF application with adhesive restorations so that the advantages of each of the materials are maintained.

CONCLUSION

The current research showed differences between chemical, microstructure, and mechanical properties of demineralized dentin and commercial SDF agents applied on its surface. The applications of SDF resulted in the formation of different crystalline phases of silver salts, an increase in the mineral:organic matter ratio, and larger crystallites of demineralized dentin. However, the use of SDF demonstrated a negative effect on the bond strength for etch-and-rise and self-etching adhesives. The immediate restoration of these treated cavities with SDF agents is not recommended.

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Clinical relevance: Immediate adhesive procedures are not recommended on teeth treated with SDF.